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# Two Unusual Occurrences of Trichomoniasis: Rapid Species Identification by PCR[▽]

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**PCR analysis in two unusual occurrences of trichomoniasis, trichomonal empyema due to *Trichomonas tenax* and *Trichomonas vaginalis* in an infant urine sample, allowed us to obtain rapid and accurate trichomonad species identification. The weak sensitivity of wet preparations and the low viability of the flagellates can be remedied by the PCR method.**

## CASE REPORTS

**Case 1.** A 3-month-old girl was brought to the hospital by her parents because of aggravated respiratory distress and difficulty in eating. Physical examination revealed fever (38°C), mild inflammatory syndrome (C-reactive protein, 22 mg/liter), diffuse sibilants, and congestion. A chest radiograph revealed thoracic distension. Urine and stool samples were sent to the laboratory. Whereas the stool analysis was negative, the urine analysis was positive for *Trichomonas vaginalis* twice (Fig. 1). PCR analysis was carried out on urine, stool, and saliva. The urine sample was found positive for *T. vaginalis*. Genital examination of the infant revealed no abnormalities: absence of erythema, indurations, or skin breakdown of the labia or perineal area. The child was successfully treated with metronidazole (30 mg/kg of body weight/day) for 3 days. The respiratory distress was managed with salbutamol aerosol, respiratory physiotherapy, and rhinopharyngeal disinfection. To explore the source of the contamination, the mother underwent a vaginal examination in a private laboratory, which was negative for *T. vaginalis*.

**Case 2.** A 33-year-old woman was admitted to the intensive cardiac surgical care unit for urgent cardiac transplantation. On day 9 (D9) postgraft, the patient became dyspneic and a chest radiograph showed a pneumothorax of the right lung. The patient was treated with vancomycin-cefotaxime after fluid drainage. As pain and dyspnea persisted, a chest scan was performed on D14 and demonstrated a right pneumothorax with pleural effusion. Pleural fluid was drained and was positive for *Corynebacterium* sp., *Prevotella oralis*, *Peptostreptococcus* sp., and *Streptococcus* sp. Antibiotherapy was switched to amoxicillin-clavulanic acid. On D19, a new chest scan showed a right hydropneumothorax with inflammatory syndrome (C-reactive protein, 335 mg/liter), and a surgical cleaning of the pleural cavity was decided upon. Microscopic analysis of the pleural liquid was positive for flagellated motile parasites identified as *Trichomonas tenax* by their size and morphology (Fig. 2). PCR was carried out on pleural fluid, bronchoalveolar fluid, saliva, sputum, and stool. Pleural fluid, sputum, and bronchoalveolar fluid were positive for *T. tenax*. The anti-infection treatment was changed to piperacillin-tazobactam and metronidazole, which were rapidly followed by the clearance of the parasite.

**Material and methods for PCR analysis.** DNA was extracted from bronchoalveolar fluid, saliva, sputum, and urine using the QIAamp DNA mini kit (Qiagen, Courtaboeuf, France) and from stool using the QIAamp DNA stool minikit according to the manufacturer's instructions, except that DNA was eluted from the spin columns with 50 μl of elution buffer, of which 5 μl was used per PCR.

Part of the 5.8S rRNA gene and the internal transcribed spacer flanking regions (ITS1 and ITS2) of trichomonads were amplified by PCR (7, 9). Amplification products were purified using the HighPure PCR product purification kit (Roche Diagnostics, Meylan, France) and were then sequenced using the BigDye Terminator cycle sequencing kit v3.1 protocol (Applied Biosystems, Courtaboeuf, France). The reaction products were run on an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Courtaboeuf, France) and analyzed with the Sequence Analysis/Seqscape v2.1 software (Applied Biosystems, Courtaboeuf, France), which was designed to automatically identify genotypes from a reference library after analysis of all known or unknown polymorphic positions. Negative and positive controls were included in the experiment.

Sequence analysis of PCR products revealed a nucleotide sequence for the positive samples completely concordant with the previously reported *T. vaginalis* sequence for case 1 and the *T. tenax* sequence for case 2 (5).

**Contribution of PCR analysis to diagnosis of trichomoniasis.** A diagnosis of trichomoniasis may be missed even by experienced biologists. Although precise morphological identification guides for trichomonads are available, the parasite is able to take amoeboid forms (4), and sometimes its internal structures are not visible (6). The main explanations for undiagnosed trichomoniasis are delayed transport, storage at 4°C, and freezing, which lead to lysis or loss of motility of the parasites. A recent study investigating the viability of *T.*

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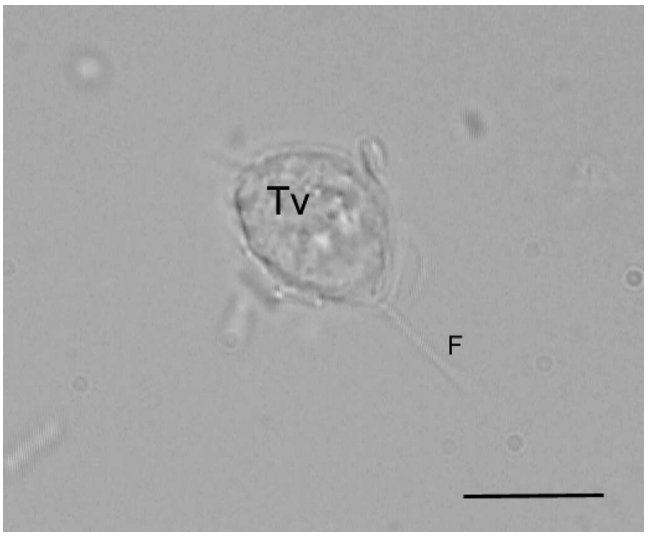


FIG. 1. *Trichomonas vaginalis* (Tv) in urine sample (direct examination). Note the anterior flagellum (F) of the organism. Magnification, ×400. Bar = 9 μm.

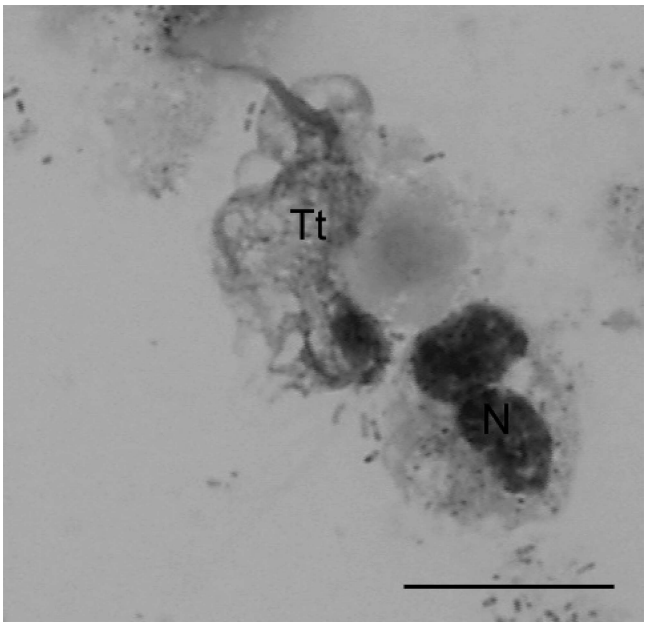


FIG. 2. *Trichomonas tenax* (Tt) and neutrophil (N) in pleural fluid (May-Grünwald-Giemsa stain). Magnification, ×1,000. Bar = 7 μm.

*vaginalis* concluded that a delay between collection and analysis of longer than 30 min could lead to false-negative results (12).

Neonates are generally infected vertically by *T. vaginalis* from the genitourinary tract at the time of delivery or by the premature rupture of fetal membranes (1), but the vaginal alkalinization that occurs at 6 weeks of age might naturally eradicate the flagellates (2). Since *T. vaginalis* is recognized as the most prevalent nonviral sexually transmitted infection worldwide (11), its detection in an infant remains an uncomfortable situation. Indeed, the discovery of a sexually transmitted organism in children can be the first indication of sexual abuse. In our case, no argument in favor of sexual abuse was found after investigation. The wet mount preparation carried out on the vaginal sample of the mother in a private laboratory was found to be negative, but we did not obtain this swab in order to confirm the result by PCR analysis.

Concerning pleural empyema with *T. tenax*, 15 cases have been reported to date (13). Usually, *T. tenax* is found as a commensal of the human oral cavity in those with poor oral hygiene. In the case of empyema, anaerobic and aerobic bacteria in addition to the trichomonads are often present and *T. tenax* eats them. The fact that some of these observations were published when PCR techniques were not available and that the delay between collection and analysis is unknown suggests a higher prevalence of this occurrence. Systematic use of PCR for the detection of trichomonads in sputum could determine if *T. tenax* is really rarely present in pulmonary tracts, and an update of epidemiological data could be made (3, 10, 14). Recently, an Egyptian study designed to compare four diagnostic methods for trichomonads, including PCR, sought *T. tenax* systematically in 200 samples of sputum from patients at risk of developing pleural empyema and established that 10% were positive by one or more methods (8). Species other than *T. tenax* can be responsible for infection of the upper respiratory tract, and their identification is achieved by PCR methods. To illustrate this, *Trichomonas hominis*, regarded as an intestinal commensal, was identified in a purulent pleural fluid (7) and *T. vaginalis*, generally common in the genitourinary tract, was recently reported in a neonatal respiratory infection (1).

**Conclusion.** PCR processes provide rapid and reliable identification of trichomonad species and avoid undiagnosed trichomoniasis related to a delayed analysis of the sample. Updating the prevalence of the different trichomonal species could also be provided by a systematic use of molecular biology to investigate trichomoniasis.

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